Pancreatic Cancer Risk and Nutrition-Related Methyl-Group Availability Indicators in Male Smokers

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Background: **Few risk factors for pancreatic cancer have been identified, with age and cigarette smoking being the most consistent. The protective effect associated with consumption of fruits and vegetables—the major dietary sources of folate—is suggestive of a role for factors influencing cellular methylation reactions; however, to our knowledge, no study has investigated this relationship. Whether biochemical indicators of methyl-group availability are associated with exocrine pancreatic cancer risk was the focus of this investigation.** ***Methods:*** **We conducted a nested case–control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of 29 133 male Finnish smokers aged 50–69 years. One hundred twenty-six subjects with incident exocrine pancreatic cancer were matched by date of baseline blood draw (±30 days), study center, age (±5 years), trial intervention group, and completion of dietary history to 247 control subjects, who were alive and free from cancer at the time the case subjects were diagnosed. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by use of conditional logistic regression. Reported *P* values are two-tailed.** ***Results:*** **Serum folate and pyridoxal-5′-phosphate (PLP) concentrations showed statistically significant inverse dose–response relationships with pancreatic cancer risk, with the highest serum tertiles having approximately half the risk of the lowest (folate: OR = 0.45; 95% CI = 0.24–0.82; *P* for trend = .009, and PLP: OR = 0.48; 95% CI = 0.26–0.88; *P* for trend = .02). An increased pancreatic cancer risk was also observed with greater exposure to cigarettes (e.g., pack-years [number of packs smoked per day × number of years of smoking], highest versus lowest quartile: OR = 2.13; 95% CI = 1.13–3.99; *P* for trend = .04).** ***Conclusions:*** **These results support the hypothesis that maintaining adequate folate and pyridoxine status may reduce the risk of pancreatic cancer and confirm the risk previously associated with cigarette smoking. [J Natl Cancer Inst 1999;91:535–41]**

Pancreatic cancer ranks eleventh for cancer incidence and fifth for cancer mortality in the United States *(1).* Compared with other European nations and the United States, Finland has a relatively high mortality rate for pancreatic cancer *(2).* Pancreatic cancer is diagnosed most often at advanced stages, and patients with this cancer have a poor survival.

Few risk factors for pancreatic cancer have been identified. Age and cigarette smoking are most consistently associated with greater risk *(3,4)* with a rapid and substantial reduction in risk following smoking cessation *(5).* Coffee consumption and alcohol consumption have been studied extensively with conflicting results, and recent reviews *(4,6)* have concluded that there is insufficient evidence to support either as a causal factor. There is evidence to support a protective role for diets high in fruits and vegetables, vitamin C, and fiber *(6),* although it remains unknown whether (or which) specific nutrients in fruits and vegetables may account for the association.

The methyl donors, methionine and choline, and the methyl cofactors, folate and vitamin B_{12}, are nutritional components involved in methylation and synthesis of DNA. Imbalances in DNA methylation may affect chromosome stability and gene expression throughout carcinogenesis *(7).* DNA hypomethylation or hypermethylation may increase the susceptibility of genes to mutations *(7,8).* In addition, hypomethylation may increase oncogene expression, and hypermethylation may silence tumor suppressor gene expression *(9,10).* Methionine, in the form *S*-adenosyl methionine, is the principal methyl donor for methylation reactions. Although methionine is an essential amino acid required in the diet of humans, a substantial proportion can be regenerated from homocysteine via methionine synthase with methyltetrahydrofolate and vitamin B_{12} as cofactors. Methylenetetrahydrofolate is used to synthesize methyltetrahydrofolate via methylenetetrahydrofolate reductase, as well as to synthesize nucleotides (purines and thymidylate) for DNA synthesis. Cigarette smoke may influence methyl-group availability by affecting folate status and interfering with vitamin B_{12} metabolism *(11,12).* Diets having lower methyl-group availability (i.e., low intake of folate and methionine and high alcohol consumption) have been associated with colorectal cancer *(13,14)* and may likewise contribute to pancreatic cancer. The protective association between fruits and vegetables, the major dietary folate sources, and pancreatic cancer *(4)* suggests that factors influencing methylation might be related to the development of this cancer. To our knowledge, no study has examined this relationship.

We investigated concentrations of serum folate (methyltetrahydrofolate), total homocysteine, and vitamin B_{12} in relation to pancreatic cancer risk. To increase the precision around homocysteine's use as a marker for methylation reactions, we also measured serum creatinine and pyridoxal-5′-phosphate (PLP, the coenzyme form of vitamin B_6 measured in serum), which have been positively and inversely associated with serum homocysteine, respectively *(15,16).* Our purpose was to determine whether nutritional and environmental factors known to influence methyl-group availability are associated with the development of pancreatic cancer.

Subjects and Methods

We conducted a nested case–control study within the Alpha-Tocopherol, Beta-Carotene Cancer Pre-

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See "Notes" following "References."

vention (ATBC) Study cohort. The ATBC Study was a double-blind, placebo-controlled, 2 × 2 factorial design, primary prevention trial that tested whether supplementation with α-tocopherol or β-carotene could reduce the incidence of cancer among male smokers *(17)*. During the period from 1985 through 1988, a total of 29 133 eligible men in southwestern Finland who were aged 50–69 years and who smoked at least five cigarettes per day were randomly assigned to receive either active supplements or placebo. Men were excluded from the study if they had a history of cancer other than nonmelanoma cancer of the skin or carcinoma *in situ*, severe angina upon exertion, chronic renal insufficiency, liver cirrhosis, chronic alcoholism, anticoagulant therapy, other medical problems that might have limited long-term participation, or current use of supplements containing vitamin E (>20 mg/day), vitamin A (>20 000 IU/day), or β-carotene (>6 mg/day).

The study was approved by the institutional review boards of both the National Public Health Institute in Finland and the U.S. National Cancer Institute. All study participants provided written informed consent prior to the study's initiation. Details of the study rationale, design, and methods have been described previously *(17)*.

Baseline Characteristics, Smoking, and Dietary Factors

The study participants completed questionnaires on general background characteristics—medical, smoking, and dietary histories during their baseline visit. Diet was assessed with the use of a self-administered dietary history questionnaire that determined the frequency of consumption and usual portion size of 276 food items during the past year; a color picture booklet was used as a guide for food items and portion size *(17)*. The questionnaire was linked to a food-composition database of the National Public Health Institute in Finland. The questionnaire was developed for the ATBC Study, and its correlation coefficients for validity and reliability ranged from .40 to .80 and from .56 to .88, respectively *(14,18)*. The diet history questionnaire was completed by 93% of the ATBC Study participants *(17)* and by 92% of the pancreatic cancer case subjects.

Case Ascertainment and Control Selection

Cases were ascertained by linkage of the cohort participants to the Finnish Cancer Registry and death certificates. The Finnish Cancer Registry provides almost 100% case ascertainment in Finland *(17,19,20)*. All relevant medical records were collected by ATBC Study personnel for reported incident pancreatic cancer cases and were reviewed independently by two study oncology experts *(17)*. Histopathologic diagnosis was assigned centrally by the study pathology review group after examining pathology and cytology specimens *(17)*. Only cases confirmed by the study physicians as incident primary malignant neoplasms of the exocrine pancreas (International Classification of Diseases, ninth revision [ICD-9] *(21)* code 157), during the period from January 1985 through December 1995, were used for the analysis. The follow-up time ranged from 7 to 10 years. Islet cell carcinomas (ICD-9 code 157.4) were excluded because their etiology may be different from that of the exocrine tumors. There were 130 confirmed exocrine pancreatic cancer cases.

Control subjects were selected from participants in the ATBC Study who were alive at the time the case subject was diagnosed and free from cancer except nonmelanoma skin cancer as of December 1995. Two control subjects were matched to each case subject by *(a)* age (±5 years); *(b)* baseline month of blood draw, to control for seasonal variation of nutrient intake and sample degradation; *(c)* completion of dietary history questionnaire, to maximize power for the dietary analysis; *(d)* study center, to control for potential differences in blood handling; and *(e)* intervention group assignment.

Of the 130 case subjects with pancreatic cancer and 260 matched control subjects, 126 case subjects and 247 control subjects were used for the statistical analyses of the serum analytes. The remaining individuals were excluded because of cracked vials and serum loss during the laboratory analyses. One hundred sixteen of the case subjects and their matched control subjects had complete dietary histories.

Assessment of Serum Biomarkers

Fasting serum samples were collected at the ATBC Study participants' prerandomization baseline visit, and samples were stored in the dark at −70 °C. The stored serum samples from the case and control subjects were sent on dry ice to the Vitamin Metabolism Laboratory at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center at Tufts University, Boston, MA, for nutrient determination.

Serum samples from the case and control subjects were analyzed for total concentrations of homocysteine, vitamin B_{12}, folate, PLP, and creatinine. Total homocysteine concentration was determined by use of high-performance liquid chromatography with fluorescence detection done as described by Araki and Sako *(22)*. Serum folate and vitamin B_{12} (cobalamin) concentrations were determined by radioassay with a commercial kit from Bio-Rad Laboratories (Richmond, CA), and PLP concentration was determined by the tyrosine decarboxylase apoenzyme method as described by Shin-Buehring et al. *(23)*. Measurement of serum creatinine levels was performed by use of the standard method *(24)*.

Specimens from the case and control subjects were handled in the same standard manner. The laboratories were blinded to case and control status. Matched serum case and control samples were analyzed consecutively as triplets. Blinded replicate quality-control phantom samples from male volunteers aged 50–69 years were placed toward the beginning and the end of each batch. The quality-control sample size was approximately 10% of each batch and the study sample. Intra-batch and inter-batch coefficients of variation percent, respectively, were 10.1 and 13.8 for serum total homocysteine, 11.7 and 13.5 for serum PLP, 6.8 and 9.0 for serum folate, 7.6 and 11.3 for serum vitamin B_{12}, and 4.3 and 4.6 for serum creatinine.

Baseline serum α-tocopherol, β-carotene, and retinol concentrations were determined in all participants during the trial by high-performance liquid chromatography *(17,25)* and used to evaluate for confounding of the main effects.

Statistical Analyses

Analyses were performed separately for nutrients from serum, foods, and supplements. Nutrients were analyzed both as continuous and as categorical variables. Variables were categorized on the basis of the distribution of the controls for the conditional logistic regression analyses. Trends of the categorical variables were tested by calculating a score variable based on the median values of each category. Categories for the smoking inhalation variable were defined as never/seldom, often, and always. Pack-years were estimated from baseline smoking history by multiplying the number of years of smoking by the average number of packs smoked per day. Smoking cessation was defined as having quit smoking for three or more consecutive follow-up visits during the trial or for 1 year.

Spearman correlations were performed to assess correspondence between the study variables. Linear regression models for serum folate, vitamin B_{12}, PLP, and total homocysteine were created to determine which other factors, including dietary nutrient estimates, were predictive of the serum markers. Natural log-transformed serum nutrients were used as dependent variables in the linear regression models, if they were not normally distributed. Dietary nutrients that were highly correlated (statistically) with energy (data not shown) were energy adjusted by use of the residual method described by Willett and Stampfer *(26)*. Log-transformed dietary nutrients were used for energy adjustment for those nutrients that were not normally distributed.

Because many of the variables had skewed distributions, the characteristics of the case and control subjects were compared by use of the nonparametric Wilcoxon rank sum test for continuous variables and chi-squared tests for proportions. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by use of conditional logistic regression. Multivariable models were developed separately for each serum nutrient and smoking variable by individually adding covariates to the model. Variables were considered confounders if they were associated with both the disease and the risk factor and if they changed the risk estimate by 10% or more. Effect modification was determined by the addition of interaction terms using the categorical trend variables to the multivariable models and by stratification. Attributable risk was estimated with Levin's formula *(27)* by determining the OR for serum folate and PLP with the most adequate serum tertile as the reference category.

All statistical analyses were performed with the use of Statistical Analytic Systems (SAS) software (SAS Institute, Inc., Cary, NC), and statistical tests were two-tailed. Because case subjects and control subjects were matched, the median values, proportions, and risk estimates (including those labeled as crude) should be interpreted as adjusted for the matching factors.

RESULTS

Selected characteristics of the case subjects and control subjects are shown in Table 1. Compared with control subjects, pancreatic cancer case subjects had statistically significantly lower levels of serum and dietary folate ($P = .03$ and $P = .03$, respectively), lower levels of serum PLP ($P = .01$), and greater pack-years of smoking ($P = .03$).

Table 1. Characteristics of case subjects and matched control subjects*

	Case subjects	Control subjects
No. of subjects	126	247
Age at case subject's diagnosis, y	64 (60–67)	63 (60–67)
Body mass index†	25.7 (23.5–28.2)	25.7 (23.6–28.2)
Smoking history		
Total cigarettes smoked/day	20 (15–25)	20 (13–25)
Years of smoking	40 (34–44)	38 (32–43)
Pack-years‡	38.5 (26–50)	35 (22–45)
Age started smoking, y	19 (16–21)	19 (17–21)
Smoke inhalation		
Never/seldom	12%	9%
Often	32%	39%
Always	56%	53%
Dietary intake		
Energy, kcal	2599 (2192–3024)	2668 (2255–3211)
Protein, g	97 (79–112)	98 (82–116)
Folate, μg	303 (239–360)	322 (265–385)
Vitamin B_{12}, μg	9.6 (7.4–12.6)	9.3 (7.4–12.4)
Vitamin B_6, mg	2.4 (1.9–2.8)	2.4 (2.0–2.9)
Vitamin C, mg	80 (59–107)	89 (62–116)
Supplement intake§		
Folic acid	6%	5%
Vitamin B_{12}	9%	7%
Vitamin B_6	11%	12%
Serum		
Folate, ng/mL	3.55 (2.86–4.27)	3.73 (3.03–4.95)
Vitamin B_{12}, pg/mL	484 (390–612)	497 (393–593)
Total homocysteine, μmol/L	10.91 (9.36–13.22)	11.07 (9.3–13.5)
Pyridoxal-5′-phosphate,‖ nmol/L	26.7 (21.2–39.0)	31.9 (22.9–45.9)
Creatinine, μmol/L	96.4 (88.4–105.2)	99.01 (91.1–106.1)

*All characteristics except age at diagnosis are baseline. Values in columns = medians (inter-quartile ranges) or proportions expressed in percents.

†Body mass index = weight in kg/height in m^2.

‡Pack-years = number of packs smoked per day × number of years of smoking.

§Nutrient range of supplements among case subjects and control subjects who took supplements, respectively: folic acid, 14–100 μg and 100–400 μg; vitamin B_{12}, 0.14–800 μg and 1–400 μg; and vitamin B_6, 0.29–800 mg and 1–400 mg.

‖The coenzyme form of vitamin B_6 measured in serum.

Approximately 90% of our case subjects and control subjects had less than adequate folate status (i.e., <6 ng/mL), with 25% having concentrations considered in the range of deficiency (i.e., <3 ng/mL) *(28)*. Fifty percent of the study sample had less than adequate pyridoxine status (i.e., <30 nmol/L) *(29,30)*. With the exception of three control subjects, our entire sample had vitamin B_{12} concentrations within the normal range (i.e., >200 pg/mL) *(28)*. Approximately 15% of our case subjects and control subjects had mild homocysteinemia (i.e., >15 μmol/L) *(31)*. Serum folate and PLP were significantly associated with their respective energy-adjusted dietary intake and supplemental intake, whereas serum vitamin B_{12} was associated with supplemental vitamin B_{12} only (data not shown).

Table 2 presents results from the multivariable conditional logistic regression models predicting cancer risk by use of the baseline serum biomarkers categorized as tertiles. Pancreatic cancer was inversely associated with both serum folate and PLP, with the highest nutrient tertiles being at half the risk compared with the lowest tertiles, and both nutrients demonstrated statistically significant trends. The addition of serum vitamin B_{12}, serum total homocysteine, and number of cigarettes smoked daily to the univariate model of serum folate strengthened folate's inverse association. Serum folate attenuated the association between serum PLP and pancreatic cancer. After we controlled for serum folate, the highest tertile of serum total homocysteine and serum vitamin B_{12} suggested nonsignificant inverse and positive associations, respectively, with pancreatic cancer. Exclusion of case subjects diagnosed early during follow-up (i.e., in the first 1, 2, 3, or even 4 years; data not shown) did not change the risk estimates. The attributable risk estimate for serum folate and PLP (lowest tertile versus highest tertile) is 0.29 and 0.26, respectively.

Table 3 presents ORs for pancreatic cancer according to smoking history. Number of cigarettes smoked daily, years smoked, and pack-years were positively associated with risk, with the highest quartile of each showing an OR close to 2.0 and with pack-years showing a significant trend across quartiles. These risk estimates were not altered by including any of the serum nutrients or other potential confounders in the models. Age at which smoking started and degree of smoke inhalation (data not shown for the latter) were not related to risk. Sixteen pancreatic case subjects (13%) and 46 control subjects (19%) quit smoking for at least 1 year during the trial, and the group had a nonsignificant lower OR for pancreatic cancer (OR = 0.64; 95% CI = 0.35–1.17).

We also evaluated the interaction between smoking exposure and nutritional status (Table 4). There were statistically significant interactions between smoking dose and levels of serum vitamin B_{12} and PLP, respectively, although the dose–response relationships for these serum nutrients within smoking strata are not consistent. Within each serum nutrient tertile, men who smoked more cigarettes daily were generally at increased risk for pancreatic cancer relative to those who smoked fewer cigarettes daily, with the exception of those in the highest serum vitamin B_{12} tertile for whom a statistically significant increased OR was observed among those who smoked the least. PLP showed a stronger and statistically significant inverse association among men with the most adequate status (highest tertile) and who smoked the least. Smoking cessation did not interact with the methyl-group-related serum nutrients (data not shown).

Finally, our study results were not altered by the addition of baseline serum α-tocopherol, β-carotene or retinol, or dietary vitamin C to our multivariable models for the serum folate, PLP, vitamin B_{12}, or total homocysteine. In addition, there was no statistically significant interaction with these baseline variables or with study intervention group (α-tocopherol or β-carotene) with the methyl-group-related serum nutrients. All the variables used for the conditional logistic regression models met the assumptions for proportional hazards.

Table 2. Crude* and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of pancreatic cancer by baseline serum nutrient tertiles among 126 case subjects and 247 matched control subjects

Serum nutrient	Serum nutrient tertile 1	2	3	*P* for trend†
Folate‡				
No. of case subjects/No. of control subjects	54/84	43/81	29/82	
Crude OR (95% CI)	1.00 (referent)	0.79 (0.47–1.33)	0.53 (0.30–0.93)	.03
Adjusted OR (95% CI)	1.00 (referent)	0.74 (0.43–1.27)	0.45 (0.24–0.82)	.009
Vitamin B_{12}§				
No. of case subjects/No. of control subjects	44/83	36/82	46/82	
Crude OR (95% CI)	1.00 (referent)	0.79 (0.46–1.36)	1.08 (0.64–1.81)	.78
Adjusted OR (95% CI)	1.00 (referent)	0.89 (0.51–1.55)	1.26 (0.73–2.17)	.42
Total homocysteine‖				
No. of case subjects/No. of control subjects	43/84	46/81	37/82	
Crude OR (95% CI)	1.00 (referent)	1.04 (0.61–1.76)	0.87 (0.49–1.53)	.58
Adjusted OR (95% CI)	1.00 (referent)	0.95 (0.55–1.63)	0.65 (0.36–1.18)	.14
PLP¶				
No. of case subjects/No. of control subjects	61/83	36/82	29/82	
Crude OR (95% CI)	1.00 (referent)	0.54 (0.31–0.95)	0.43 (0.24–0.77)	.006
Adjusted OR (95% CI)	1.00 (referent)	0.61 (0.34–1.07)	0.48 (0.26–0.88)	.02

*Crude ORs were adjusted for the matching variables (age, month of blood draw, completion of dietary questionnaire, study center, and intervention group).

†Two-sided *P* values.

‡Serum folate was also adjusted for serum vitamin B_{12}, serum total homocysteine, and number of cigarettes smoked daily. Serum folate tertiles: 1 (≤3.33 ng/mL), 2 (>3.33 ng/mL and ≤4.45 ng/mL), and 3 (>4.45 ng/mL).

§Serum vitamin B_{12} was also adjusted for serum folate. Serum vitamin B_{12} tertiles: 1 (≤427 pg/mL), 2 (>427 pg/mL and ≤550 pg/mL), and 3 (>550 pg/mL).

‖Serum total homocysteine was also adjusted for serum folate. Serum total homocysteine tertiles: 1 (≤9.99 μmol/L), 2 (>9.99 μmol/L and ≤12.51 μmol/L), and 3 (>12.51 μmol/L).

¶PLP = pyridoxal-5′-phosphate. Serum PLP was also adjusted for serum folate. Serum PLP tertiles: 1 (≤26.34 nmol/L), 2 (>26.34 nmol/L and ≤39.46 nmol/L), and 3 (>39.46 nmol/L).

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) of pancreatic cancer by quartile of smoking habits among 126 case subjects and 247 control subjects

	Smoking variable quartile 1	2	3	4	*P* for trend*
Cigarettes smoked daily†					
No. of case subjects/No. of control subjects	25/63	56/113	18/33	27/38	
OR (95% CI)	1.00 (referent)	1.35 (0.75–2.43)	1.43 (0.68–3.02)	1.90 (0.91–3.95)	.10
Smoke-years‡					
No. of case subjects/No. of control subjects	27/65	29/59	34/70	36/53	
OR (95% CI)	1.00 (referent)	1.12 (0.58–2.15)	1.30 (0.66–2.56)	2.07 (0.95–4.50)	.09
Pack-years§					
No. of case subjects/No. of control subjects	24/66	33/60	27/67	42/54	
OR (95% CI)	1.00 (referent)	1.59 (0.85–2.99)	1.15 (0.60–2.20)	2.13 (1.13–3.99)	.04
Age started smoking‖					
No. of case subjects/No. of control subjects	40/71	26/56	35/70	25/50	
OR (95% CI)	1.00 (referent)	0.81 (0.43–1.51)	0.89 (0.51–1.57)	0.92 (0.48–1.75)	.80

*Two-sided *P* values.

†Cigarettes smoked daily quartiles: 1 (≤13), 2 (>13 and ≤20), 3 (>20 and ≤25), and 4 (>25).

‡Smoke-years = number of years of smoking; smoke-year quartiles: 1 (≤32), 2 (>32 and ≤38), 3 (>38 and ≤43), and 4 (>43).

§Pack-years = number of packs smoked per day × number of years of smoking; pack-year quartiles: 1 (≤22), 2 (>22 and ≤35), 3 (>35 and ≤45), and 4 (>45).

‖Age started smoking quartiles: 1 (≤17 years), 2 (>17 years and ≤19 years), 3 (>19 years and ≤21 years), and 4 (>21 years).

DISCUSSION

To our knowledge, this study is the first to examine and observe statistically significant, inverse associations between serum concentrations of folate and PLP and pancreatic cancer risk. The inverse dose–response relationships observed for serum folate and PLP showed twofold risk reductions for those in the highest tertiles. These findings were independent of other risk factors, including smoking history and diet. In addition, there were statistically significant interactions between smoking dose and both serum vitamin B_{12} and serum PLP, respectively.

An important strength of our study lies in its large prospective nature, with a greater number of cases providing sufficient power to detect smaller differences in risk factors, compared with most previous studies *(4)*. We eliminated recall bias by obtaining the biochemical sample and other risk factor exposure data at baseline, before pancreatic cancer was diagnosed. Our study also has internal consistency; both the case and the control

Table 4. Odds ratios (ORs) and 95% confidence intervals (CIs) of pancreatic cancer by baseline serum nutrient tertile and number of cigarettes smoked daily among 126 case subjects and 247 matched control subjects

Serum nutrient	No. of cigarettes smoked per day	Serum nutrient tertile 1	2	3	*P* for trend*
Folate†	⩽20				
	No. of case subjects/No. of control subjects	38/58	26/64	17/54	
	OR (95% CI)	1.00 (referent)	0.58 (0.30–1.10)	0.44 (0.21–0.93)	.71
	>20				
	No. of case subjects/No. of control subjects	16/26	17/17	12/28	
	OR (95% CI)	0.95 (0.42–2.13)	1.32 (0.56–3.07)	0.54 (0.24–1.24)	
Vitamin B_{12}‡	⩽20				
	No. of case subjects/No. of control subjects	28/66	18/62	35/48	
	OR (95% CI)	1.00 (referent)	0.69 (0.34–1.37)	2.23 (1.14–4.36)	.005
	>20				
	No. of case subjects/No. of control subjects	16/17	18/20	11/34	
	OR (95% CI)	2.01 (0.90–4.48)	2.33 (1.00–5.45)	0.80 (0.34–1.92)	
Total homocysteine§	⩽20				
	No. of case subjects/No. of control subjects	24/59	34/58	23/59	
	OR (95% CI)	1.00 (referent)	1.28 (0.65–2.51)	0.70 (0.33–1.48)	.74
	>20				
	No. of case subjects/No. of control subjects	19/25	12/23	14/23	
	OR (95% CI)	2.13 (0.97–4.70)	1.10 (0.47–2.52)	1.09 (0.47–2.52)	
PLP‖	⩽20				
	No. of case subjects/No. of control subjects	44/59	23/55	14/62	
	OR (95% CI)	1.00 (referent)	0.53 (0.27–1.02)	0.30 (0.14–0.64)	.02
	>20				
	No. of case subjects/No. of control subjects	17/24	13/27	15/20	
	OR (95% CI)	0.81 (0.37–1.75)	0.66 (0.30–1.49)	0.96 (0.41–2.24)	

*Two-sided *P* values.

†Serum folate was adjusted for serum vitamin B_{12} and total homocysteine. Serum folate tertiles: 1 (⩽3.33 ng/mL), 2 (>3.33 ng/mL and ⩽4.45 ng/mL), and 3 (>4.45 ng/mL).

‡Serum vitamin B_{12} was adjusted for serum folate. Serum vitamin B_{12} tertiles: 1 (⩽427 pg/mL), 2 (>427 pg/mL and ⩽550 pg/mL), and 3 (>550 pg/mL).

§Serum total homocysteine was adjusted for serum folate. Serum total homocysteine tertiles: 1 (⩽9.99 μmol/L), 2 (>9.99 μmol/L and ⩽12.51 μmol/L), and 3 (>12.51 μmol/L).

‖PLP = pyridoxal-5′-phosphate. Serum PLP was adjusted for serum folate. Serum PLP tertiles: 1 (⩽26.34 nmol/L), 2 (>26.34 nmol/L and ⩽39.46 nmol/L), and 3 (>39.46 nmol/L).

subjects were derived from the same study cohort, thus eliminating control selection bias. Consistent with other studies and despite not having a nonsmoking comparison group, our study demonstrated increasing risk and a positive dose–response relationship for pancreatic cancer with greater smoking. Our measurement of serum nutrient concentrations better reflects absorption and biologically active dose than self-reported intake. Finally, the case subjects had statistically lower baseline dietary folate intake than the control subjects, which is in accordance with our serum folate results.

Mechanisms by which the methyl-group-related serum nutrients may influence carcinogenesis remain speculative. DNA hypomethylation, through epigenetic, nongenotoxic events *(9)*, could result from folate inadequacy. Thus far, only transcriptional silencing of the wild-type p16 tumor suppressor gene through regional hypermethylation of the gene's 5′-CpG islands has been observed in 14%–21% of pancreatic tumors *(32)*. Higher total homocysteine concentrations may prevent hypermethylation by less regeneration of methionine for methylation reactions and possibly account for our observed risk reduction with higher total homocysteine concentrations (after controlling for folate status). Hypermethylation could lead to decreased expression of tumor suppressor genes and increased susceptibility to carcinogens *(7)*, possibly accounting for the positive association with serum vitamin B_{12} and its interaction with smoking dose. Cigarette smoke-related cyanide, organic nitrites, and nitrous oxide may inactivate vitamin B_{12} and methionine synthase and thus interfere with vitamin B_{12}'s function *(12,33)*. Heavy smokers have higher serum concentrations of the coenzymatically inactive form of vitamin B_{12}, cyanocobalamin *(33)*, which could result in decreased remethylation of methionine, decreased levels of *S*-adenosyl methionine, and less DNA methylation in tissues.

Alternatively, inadequate folate or pyridoxine status could result in less methylenetetrahydrofolate available for methylation of deoxyuridylate to deoxythymidylate, misincorporation of uracil for thymine in DNA, and a greater potential for chromosome strand breaks *(34)* and/or impaired DNA excision repair *(35)*. PLP is required as a coenzyme for the synthesis of methylenetetrahydrofolate. Studies that have demonstrated a protective association between the homozygous recessive thermolabile methylenetetrahydrofolate reductase genotype, C (cytosine) → T (thymine) substitution at base pair 677 (alanine to valine) and colon cancer *(36,37)* and our observed reduced risk associated with more adequate folate and PLP status may support this mechanism.

Deficiencies in both folate and pyridoxine have been shown to impair pancreatic exocrine function in rats *(38–42)*. This situation could theoretically lead to incomplete digestion of food, greater duodenal cholecystokinin release, and stimulation of pancreatic enzyme production, hypertrophy, and hyperplasia, thereby increasing the susceptibility of the pancreas to carcinogens. Chronic hypercholecystokininemia has been shown to enhance pancreatic carcinogenesis in animals *(43–*

46). In addition, animals treated with an inhibitor of cellular methylation reactions, ethionine, develop acute hemorrhagic pancreatitis *(47–49)* as a consequence of autolytic destruction of the pancreas *(50)*, and chronic pancreatitis has been associated with increased pancreatic cancer risk *(51–54)*.

Our having studied male Finnish smokers raises the question of generalizability to other populations. Smokers are a high-risk population for pancreatic cancer because cigarette smoke contains chemicals that are likely pancreatic carcinogens *(3)*. Nitrosamines produced during cigarette smoking can induce G (guanosine) → A (adenosine) transitions at the second nucleotide of a GG pair, which is the most common alteration found in pancreatic cancer K-ras mutations *(55)*. The overwhelming effect of the smoke-related carcinogens to which heavy smokers are exposed likely explains the lack of a dose–response relationship observed with serum PLP among those with greater smoking dose (Table 4). Cigarette smoke, however, has been attributed to 29% of pancreatic cancer deaths in males *(56)*, such that other exposures must contribute to the development of the disease.

The fact that the majority of our study sample had less than adequate folate and pyridoxine status is likely relevant to our findings. Lower folate status has been associated with risk for neural tube defects *(57)*. By contrast, Finland has a relatively low incidence of neural tube defects (five of 10 000 births), approximately half of that of the United States (10 of 10 000 births) *(58)*. International differences in the incidence of neural tube defects are likely influenced by genetic predisposition and diet. Our population's low folate and PLP concentrations most likely reflect their smoking and/or dietary habits. Cigarette smokers tend to have poorer nutritional status for these vitamins *(11,12, 29)* and may have greater requirements for them than nonsmokers. In our study of smokers, 29% and 26% of the pancreatic cancer cases may have potentially been prevented by improving the folate and pyridoxine status of those in the lowest serum tertiles, respectively, based on the attributable risk estimates. In addition, the protective relationships of these nutrients tended to be greater among those who smoked less. Adequate folate and pyridoxine status appeared to decrease the susceptibility to pancreatic cancer in the present study—a finding that should be confirmed in other smoking populations and in nonsmokers.

Other limitations of our study are that more adequate folate and pyridoxine status may reflect a healthier lifestyle or health status in general and the adjustment for serum folate in the other serum nutrient models. There could be other unmeasured correlates to more adequate nutritional status not controlled for in our analysis. In addition, poorer folate or pyridoxine status could be a marker for subclinical disease, particularly as the latency of pancreatic cancer is unknown and it is most often diagnosed at advanced stages; however, our observation of similar associations after excluding early case subjects argues against this. The potential confounding effect of other unmeasured health status-related risk factors needs further investigation. Finally, although serum folate may be interrelated with serum vitamin B_{12}, total homocysteine, and PLP in the causal pathways for disease, adjustment for serum folate in these models (or for serum total homocysteine and vitamin B_{12} in the folate model) either strengthened or slightly attenuated the crude associations, suggesting independent associations and perhaps multiple mechanisms by which these factors may be contributing to our observed associations.

In conclusion, we found statistically significant reduced risks for cancer of the exocrine pancreas associated with more adequate folate and pyridoxine status in a prospective cohort of older male smokers. Dose–response relationships were evident. Level of cigarette smoking was also positively related to cancer risk. The level of folic acid grain fortification in the United States has been estimated to reduce the risk of neural tube defects by 22%–26% in women with marginal folate status *(59)* and, in view of our study findings, could conceivably have an impact on exocrine pancreatic cancer incidence in persons with marginal folate status. The results from this observational study support the hypothesis that maintaining adequate folate and pyridoxine status may reduce the risk of pancreatic cancer and confirm the risk associated with cigarette smoking. Additional studies are needed to determine if the observed associations reflect cause-and-effect relationships.

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NOTES

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